BEHAVIORAL MONITORING OF *MICROPLITIS CROCEIPES*, A PARASITOID WASP, FOR DETECTING TARGET ODORANTS USING A COMPUTER VISION SYSTEM

S. L. Utley, G. C. Rains, W. J. Lewis

ABSTRACT. *Microplitis croceipes (Cresson) (Hymenoptera: Braconidae) are parasitoid wasps capable of being trained to respond to target odors. One such response is known as area-restricted searching, and several wasps exhibiting area-restricted searching within the same area is known as crowding. A computer vision system consisting of a laptop computer, web camera, and software package (Visual Cortex) was assembled to objectively quantify the crowding behavior of M. croceipes. The system was able to measure the crowding behavior of five female M. croceipes hand-trained to detect 3-octanone. Further, the system was able to distinguish between the crowding response of trained M. croceipes exposed to 5.5 and 1.1 mg L^{-1} of 3-octanone and a control within 20 s.*

Keywords. *3-octanone, Biological sensor, LabView, Machine vision, Visual Cortex, Volatile chemical detection.*

Microplitis croceipes, a parasitoid wasp, hand-trained to and placed in the presence of a target odor, can individually exhibit several distinct behaviors including: coiling, antennating, head sticking, and area-restricted searching (Takasu and Lewis, 1993; Wäckers et al., 2002; Olson et al., 2003). These behaviors were interpreted as either indicating a positive (odor detected) or negative (odor not detected) response by visual assessment. However, it was very difficult to objectively quantify a wasp's response to an odor. A system was needed to objectively make quantifiable measurements of the behavioral responses.

Previous attempts at developing a volatile odor detector exploited hand-trained, 2 day old, starved female wasps, and their desire to seek out food while ignoring phototropic and anti-geotropic instincts (Tertualiano et al., 2004). The movement of *M. croceipes* into a dark hole while seeking out an odor source was detected using an infrared beam. A response was considered positive if the wasps entered the hole within 10 s. Eighty-eight percent of the conditioned females exhibited a positive response when presented with the target odor. However, large variability in observed response times reduced the reliability and robustness of *M. croceipes* when used to identify target volatile chemicals. To harvest the sensitivity and specificity available from the wasp conditioned to a target odor, the large variability in response times must be reduced.

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An investigation was proposed to look for quicker, more reliable, and easily detectable behavioral responses than those offered by previous methods. A novel approach for analyzing an insect colony's spatial behavior through the use of a computer vision system was tested and implemented (Balch et al., 2001). The system was capable of simultaneously tracking hundreds of social insects (ants and bees) and recognized individual and colony behaviors. It also utilized a combination of computer vision techniques including Carnegie Mellon's CORAL Group Color Machine Vision (CMVision) algorithms, which were used to seek out insect body colors and frame differencing for identifying moving pixels. The moving pixels were grouped and classified to follow moving objects, such as insects (Balch et al., 2001).

A similar approach was taken during this study utilizing *M. croceipes*. When multiple wasps were contained together and responded individually with area-restricted searching, an emergent group behavior called crowding occurred. Crowding lent itself to detection and quantification through the use of a computer vision system, allowing for digital image acquisition and analysis.

Normalizing the pixel values helped increase image contrast for those images not containing pixels with values equal to 0 and/or 2^N - 1, where N is equal to the image resolution. Equation 1 describes the normalization of a single pixel value:

$$P'(x,y) = (2^N - 1) \cdot \left(\frac{P(x,y) - P_{MIN}}{P_{MAX} - P_{MIN}} \right) \qquad (1)$$

where $P'(x, y)$ is the normalized pixel value calculated for location (x, y) using the image resolution (N), the current pixel value at location (x, y), and the maximum (P_{MAX}) and minimum (P_{MIN}) pixel values currently contained within the image.

Creating an image with greater contrast through pixel normalization allowed for easier edge detection and object identification through binary segmentation. Binary segmen–

tation used a single threshold value for dividing each pixel within an image into one of two classifications (A and B), resulting in the creation of a binary image (Shapiro and Stockman, 2001). For example, all pixels of value lower than that of the threshold were placed into class A, in which all members were represented as black with a value of zero; all pixels of value equal to or higher than the threshold were placed into class B, in which all members were represented as white with a value of one.

Normalization and binary segmentation processes were applied to a selected region of interest (ROI) through the use of an image mask. Analogous to a stencil, the image mask covered any part of the image to be ignored and exposed the area to be viewed, studied, modified, etc. Mask application allowed for the focusing of processing efforts only on the ROI.

Objectives

The objective of this study was to develop and explore the viability of utilizing a computer vision system to objectively quantify the behavioral response of *M. croceipes* when trained by hand (not by an automated or mass training system) to detect 3-octanone, a volatile chemical compound associated with fungal pathogens.

Materials and Methods

Computer Vision System

A computer vision system, consisting of a camera, testing stage, computer, and software, was created in order to objectively observe the crowding behavior of *M. croceipes* (fig. 1).

Hardware

The computer vision system consisted of two major hardware components; a camera and a laptop computer. A Logitech QuickCam Zoom color web camera (Logitech, Inc., Fremont, Cal.) was used to acquire bitmap images of the insects. The Logitech QuickCam Zoom provided a low-cost alternative to more expensive imaging systems and up to a 640 × 480 pixel resolution. For this study, a Sony Vaio laptop computer (model PCG-GRT100, 2.4 GHz Pentium 4 processor, 512 M RAM, Microsoft Windows XP Home) was used to save and process the images acquired with the camera. The Logitech camera interfaced with the computer through USB.

Software

Software to acquire and process images was developed in-house using LabView 6.1 (National Instruments Corp., Austin, Tex.) and Parente's LabView WebCam Library (Parente, 2003). The software developed with LabView was named Visual Cortex. Visual Cortex provided a method to perform several tasks related to observing and analyzing

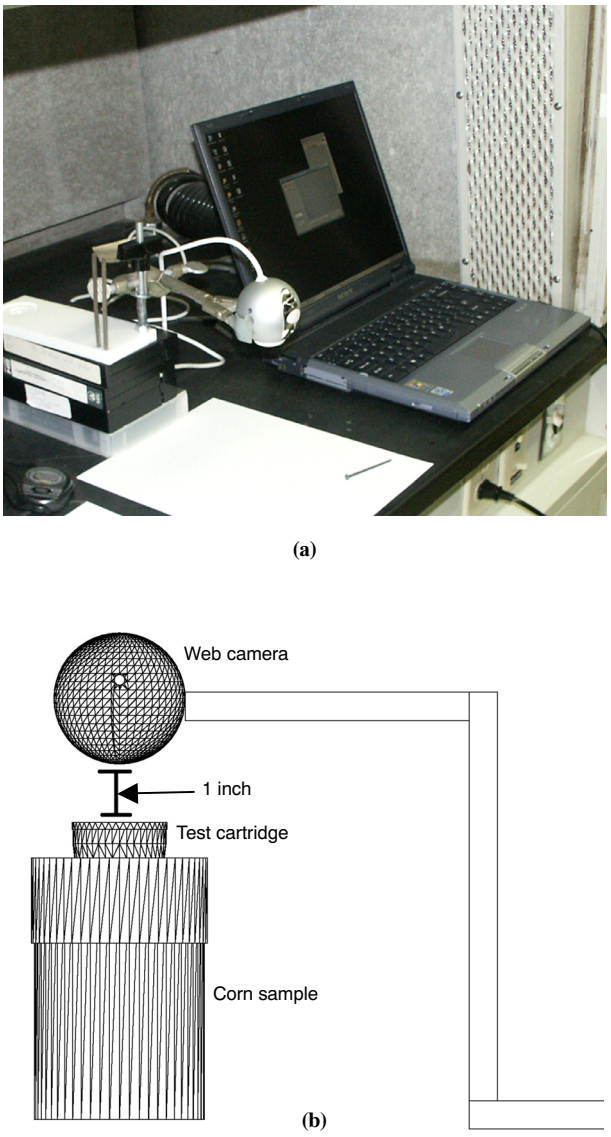


Figure 1. A computer vision system was created for observing the crowding behavior of female *Microplitis croceipes* within a test cartridge. (a) The system was used under a fume hood and consisted of a camera, testing stage, computer, and software. (b) The distance from the tip of the camera to the test cartridge top was 1 inch. This distance allowed for viewing the inside surface of the cartridge.

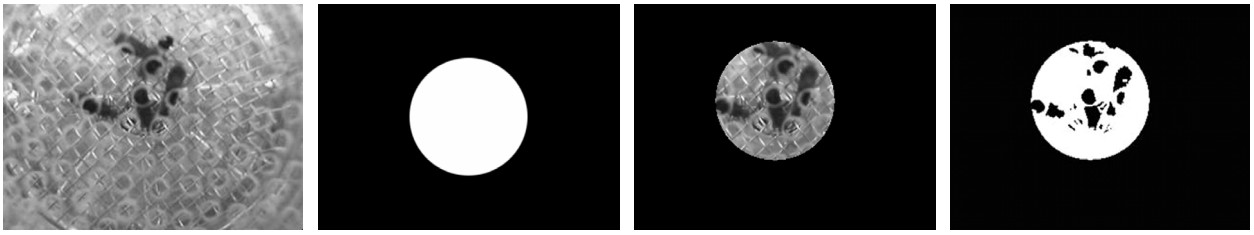


Figure 2. Image processing sequence. The original (a) is masked with a 320 y 240 pixel mask of the user's choice (b). The mask is given an offset to select an ROI for processing (c). The values of the pixels within the ROI are normalized and then processed using binary segmentation (d).

insect crowding behavior including: taking single snapshots, converting snapshots from color to gray-scale, capturing time-stamped still pictures, extracting time-variant information from still pictures using image processing, and capturing and analyzing insect behavior in real-time. The basic image manipulation and analysis techniques forming the core processes that allow Visual Cortex to analyze the crowding behavior of insects were masking, normalizing, creating histograms, and binary segmentation (fig. 2).

The core processes were utilized in both the time-stamped still image and real-time image processing features. The captured image was first masked using a 320 240 pixel mask to select a region of interest (ROI). The pixel data within the ROI was normalized and segmented, resulting in a binary image. The number of black pixels within the ROI were counted and divided by the total number of pixels within the ROI. The time-variant percent black pixel data were integrated using the trapezoidal rule (eq. 2) to produce time-variant integration curves. The integration filters the data, allowing for easier interpretation of the wasp behavior, and provides a measure of the accumulative response of the wasps:

$$I_1 = I_0 + \frac{h}{2} \cdot \left(y_0 + y_1 \right) \qquad (2)$$

where

I_1 = new integration value
I_0 = previous integration value
h = current time (t_1) - previous time (t_0)
y_1 = black pixel value for t_1 (%)
y_0 = black pixel value for t_0 (%).

Real-time analysis was provided, allowing a user to capture and process images. The activity was quantified and displayed graphically on screen. At the time of this publication, the user was left to determine when a threshold had been crossed to indicate a positive response.

Time-stamped images were captured every 250 ms and analyzed later (some variation is present in the capture interval lengths due to computer latency). The images were saved to a directory, and the last ten directories used were filed. This functionality was used extensively in this study so that records of the wasp behavior could be filed.

A virtual instrument (VI) was created to analyze the captured time-stamped still shots. This VI allows a user to browse a data disk for recorded images and set masking and threshold parameters. Additionally, it records the number and percentage of black pixels within the ROI and the resulting cumulative integration for the time-variant stills to a file of the user's choice.

Experimental Procedures

Insects

M. croceipes, a parasitoid wasp, was used for this investigation; females were used to ensure application of findings from previous studies. The larval hosts used for rearing were *Heliothis zea* (Boddie) (Lepidoptera: Notuidae), as described by Lewis and Burton (1970). The breeding stock was provided with water and honey and kept in a Plexiglas cage (30 × 30 × 17 cm) at 28°C, 50% to 70% RH, and a L16:D8 photocycle. Test specimens were 2 day old females given only water from time of emergence and no oviposition experience. For each test, seven females were trained, five of which were randomly selected and used for testing.

Training Procedure

Seven female *M. croceipes* were conditioned to associate 3-octanone ($C_8H_{16}O$, 1.5 mm Hg vapor pressure) with food. All training procedures were performed under a fume hood in the USDA-ARS Biological Control Laboratory in Tifton, Georgia. A fluorescent ring light (Luxo Corp., Elmsford, N.Y.) was used to lure wasps in the event of escape. Since escapes were common, seven wasps were trained, but only five were tested.

An odor delivery stage (fig. 3) was prepared for each group trained. First, a Whatman filter disc (Cat. No. 1103323, Grade 3, 2.3 cm, Whatman Intl. Ltd., Maidstone, U.K.) was loaded with a 10 µL aliquot of 3-octanone/dichloromethane (1:16) solution and allowed to evaporate for 1 min. Next, the filter disc was placed in a glass Petri dish (1.7 height × 5.3 cm diameter, Kimax USA), which was then covered with a piece of aluminum foil (12 × 12 cm). The head space within the covered dish was allowed to build for 1 min, during which time a piece of filter paper (2 × 2 mm) was placed in the center of the aluminum foil covering and saturated with 50% sucrose water solution. Last, a push pin was used to create six holes in a tight circular pattern around the sucrose water saturated filter paper.

Seven wasps were captured from their rearing cage and placed in separate vials. In order, each wasp was removed from its vial using a pair of forceps and individually allowed to feed on the sucrose solution for 10 s. The odorant emitted around the filter paper passed over their antennae while feeding (3-octanone at 5.5 mg L^{-1}). After feeding, each wasp was placed back in its vial. The process was repeated so each wasp was allowed to feed for three 10 s intervals with approximately 60 s between each feeding (Tertuliano et al., 2004).

Sample Preparation

Three corn sample preparations were used for testing. The preparations were named blank, control, and test. Corn was utilized as a background odor, potentially giving insight into the capabilities of utilizing *M. croceipes* for the detection of toxins present in large grain stores. The researcher's hands were washed with soap (Sparkleen 1, Fisherbrand Scientific Co., Pittsburgh, Pa.) and water prior to creating all samples. The mouth of the jar for each sample was covered with a 12 × 12 cm piece of aluminum foil and shaken for 15 s, subsequently creating small dimpling in the foil covering.

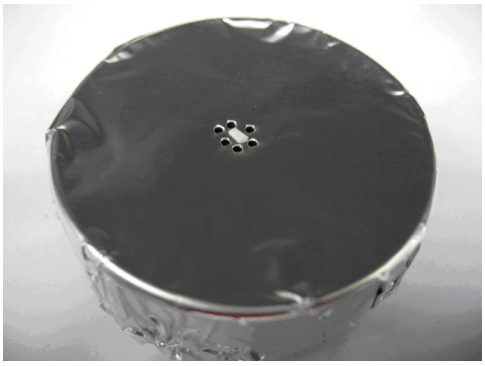
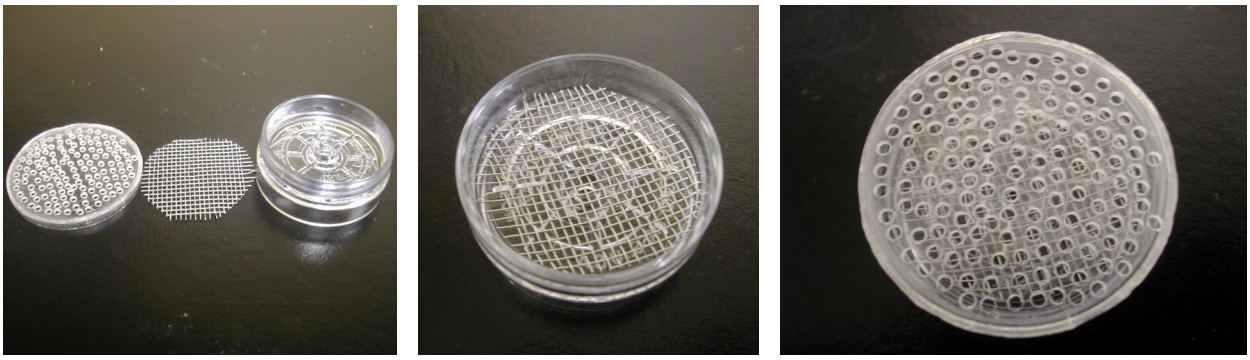


Figure 3. Odor delivery stage used during training. A filter disc was placed in a glass Petri dish and covered with aluminum foil. A piece of filter paper soaked in sucrose solution was placed in the center of the foil, and holes were made around it to allow the wasps to feed while smelling the target odor.



(a) (b) (c)

Figure 4. Test cartridge. (a) The test cartridge was composed of a top from a Millipore PetriSlide, a wire mesh disc, and part of a Millipore aerosol analysis monitor. (b) The mesh disc was placed in the body of the cartridge to prevent *M. croceipes* from escaping through the bottom. (c) The top fit onto the body and prevented *M. croceipes* from flying away while providing adequate ventilation.

Blank corn samples consisted of a 240 mL Mason jar with 150 mL (120 g) of whole-kernel feed corn. Control samples were created from existing blank samples. The foil covering of the blank sample was removed, and a Whatman filter disc was placed on top of the corn using a pair of forceps; the filter disc was pushed to the bottom of the corn using a separate pair of forceps before recovering the jar. After shaking, the sample was set aside to allow the head space over the corn to build for 5 min. Test samples were created from control samples. A Whatman filter disc was loaded with an aliquot of 3-octanone/dichloromethane solution on a glass dish and allowed to dry for 1 min. The foil covering of the control sample was removed, and the glass dish was used to drop the disc onto the top of the corn. A separate pair of forceps was used to push the odorous filter disc to the bottom of the corn before recovering the jar. After shaking, the sample was set aside to allow the head space over the corn to build for 5 min.

Cartridge Preparation

Cartridges were observed while empty and while containing five *M. croceipes*. An empty cartridge placed over a blank corn sample was defined as a blank treatment. A cartridge containing five wasps placed over a control corn sample was defined as a control treatment. A cartridge containing five wasps placed over a test corn sample was defined as a test treatment.

The cartridge was composed of three parts (fig. 4). The body of the cartridge was part of a Millipore aerosol analysis monitor (Millipore Corp., Billerica, Mass.). The top was a lid for a Millipore PetriSlide modified to fit the body and thoroughly perforated with small holes to allow for sufficient ventilation. A wire mesh disc was placed in the bottom of the body to prevent the wasps from escaping through the inlet where the target odor entered the cartridge.

Before use, each cartridge was thoroughly cleaned with Sparkleen soap and water and dried. After drying, cleaning was continued by sweeping a 10 L min^{-1} air stream over all surfaces of the cartridge for approximately 15 s. The cartridge was placed upside down in a clean area under the fume hood. Each wasp was removed from its vial using a pair of forceps and gently scraped off the forceps into the cartridge using the body of the container. Five wasps were placed individually into the cartridge.

Testing

Visual Cortex was used for comparing the behavior of five hand-trained female *M. croceipes* when presented with the air from the headspace of the prepared corn samples. Data were taken for three different concentrations of 3-octanone masked with a background odor of whole-kernel corn. The quantities of 3-octanone/dichloromethane solutions used to impregnate the filter disc were: 10 µL of a 1:16 solution (0.5 mg of 3-octanone; 5.5 mg L^{-1}), 2 µL of a 1:16 solution (0.1 mg of 3-octanone; 1.1 mg L^{-1}), and 10 µL of a 1:842 solution (0.01 mg of 3-octanone; 111 µg L^{-1}). Concentration levels were chosen at ranges close to the level of wasp conditioning (5.5 mg L^{-1}). Actual amounts of fungal odors found in corn were unknown at the time of this study. For each concentration, five replications of blank, control, and test treatments were recorded (table 1).

Testing was performed under the same fume hood as training. The area was divided into six sections, using five clean pieces of letter paper (216 × 279 mm) as markers. The sections provided dedicated areas for placing test specimens and clean objects, odor preparation, training, and testing. The laptop, camera, and samples were all placed under the fume hood. The laptop was placed in the front corner of the fume hood with the camera mount positioned approximately 15 cm to the left of the keyboard. During testing, all light sources within the room except the overhead fluorescence room

Table 1. Treatment layout: blank (no odor, no wasps), control (no odor, five wasps), and test (3-octanone, five wasps) treatments were each replicated five times for the 0.5, 0.1, and 0.01 mg dosage levels. Twelve observations (obs) occurring at multiples of 5 s were extracted from each replication (reps).

Dosage (mg)	Treatment		
	Blank	Control	Test
0.5	5 reps	5 reps	5 reps
	12 obs/rep	12 obs/rep	12 obs/rep
	60 total obs	60 total obs	60 total obs
0.1	5 reps	5 reps	5 reps
	12 obs/rep	12 obs/rep	12 obs/rep
	60 total obs	60 total obs	60 total obs
0.01	5 reps	5 reps	5 reps
	12 obs/rep	12 obs/rep	12 obs/rep
	60 total obs	60 total obs	60 total obs

lights were turned off or covered, resulting in an average light intensity of 295 lux at the top of the cartridge. The Logitech camera was placed so that the tip of the camera was approximately 2.5 cm (1 in.) above the top of the test cartridges (i.e., 1.4 cm = 125 pixels).

The following procedure was used to obtain the blank, control, and test observations for each set of wasps:

1. Hand-train seven wasps.
2. Prepare empty cartridge and blank corn sample.
3. Poke hole in aluminum foil covering with a 7-penny nail.
4. Center cartridge over new hole, and center jar beneath camera.
5. Use Visual Cortex to capture still pictures of cartridge every 250 ms for 60 s.
6. Remove cartridge from top of aluminum foil and place in clean area.
7. Wash hands, prepare control corn sample, and place sample in clean area.
8. Place five female wasps in cartridge.
9. Repeat steps 3 through 6 using control corn sample.
10. Prepare test corn sample and place in odor preparation area.
11. Wash hands and repeat steps 3 through 5 using test corn sample.
12. Discard test specimens, foil covering, and jar contents.
13. Wash cartridge and all glassware.

Image Analysis

All images collected were analyzed with Visual Cortex. For each set of pictures, a black 320 × 240 pixel TIF image containing a centered 125 pixel diameter white circle was used as a mask. The image mask was given an *X* and *Y* offset to center the white circular area over the inlet of the cartridge. By combining the original image and the mask with an AND operation, the ROI was set as the area contained within the white circular area. The ROI set by the mask corresponded to a 1.4 cm diameter circular region centered on the cartridge odor inlet. After masking the image, pixels contained within the ROI were normalized (eq. 1) to increase image contrast.

After initial studies of histograms collected from sample images, a gray-scale threshold of 70 was chosen as the optimal value for separating wasp body pixels (dark) from background pixels within the ROI (light). This threshold was used for binary segmentation (pixels values <70 forced to 0; pixels values >70 forced to 1). Visual Cortex was able to calculate the percent of total pixels (%B) and that were black within the ROI for each set of images. Additionally, it integrated the percentage values with respect to time ($\int \%B dt$).

Initial analysis revealed large variations among the 15 blank treatments. This suggested that some response curves were inherently offset due to larger numbers of black pixels within the ROI not representing wasp body mass (i.e., background noise). To remove the effects of these variances, the control and test treatment data were calibrated using a variation of image differencing. From each set of images corresponding to single tests, one image was selected in which the wasps contained within the cartridge were not searching within the ROI. This image was used to measure the amount of black pixel noise (not representing wasp body mass) present within the ROI throughout the 60 s test period. The image was masked, normalized, and segmented like all the other images processed during this study. The percentage of black pixels within the ROI was recorded for each image selected and analyzed and then used to create calibration curves for each treatment. Since the lighting within the test area and cartridge positioning would not have changed during the 60 s testing period, it was assumed that the same amount of black pixels within the ROI not contributing to the measurement of the crowding response would have remained constant throughout all the images for that single treatment. The time values recorded for each test were copied to a spreadsheet, and the percent black pixel values extracted from their corresponding calibration images were copied next to them, repeating the value for each time. The data were then integrated using the trapezoidal rule function (eq. 2) within LabView to create 30 (5 reps × 2 treatments × 3 doses) new integration curves to be used for calibration. The newly created calibrations were then subtracted from their corresponding treatment response curves.

Microsoft Excel was used to compile, average, and graph the approximately 256 integration values and their corresponding time stamps for the five replications per treatment within each concentration. The standard deviation was calculated for the integration values at 5 s intervals (excluding zero). Confidence intervals were calculated using the resultant standard deviation values, $\alpha = 0.05$ and $n = 5$, where α is the probability of rejecting the null hypothesis and n is the sample size.

An ANOVA statistical analysis of the data was performed using a general linear model (SAS). There were three dosage levels (0.5 mg/5.5 mg L^{-1}, 0.1 mg/1.1 mg L^{-1}, and 0.01 mg/111 µg L^{-1}), three treatments (blank, control, test), five replications of each dosage/treatment pair (15 total), and 12 observations from each replication (multiples of 5 s) to create a total of 540 observations analyzed with the general linear model (GLM). The 15 blank treatment replications (180 observations) were analyzed to determine if each was statistically the same. The remaining 30 calibrated replications (15 controls, 15 tests) were analyzed by dosage and next by treatment to determine if either factor had significant effect on the mean response.

Results and Discussion

Comparison of Blank Treatment Replications

There were significant differences between the 15 blank replications (degrees of freedom (d.f.) = 14, number of observations (n) = 180, probability (p) < 0.0001), indicating that the number of black pixels measured within the ROI of the empty cartridges varied significantly, even though the number of pixels was very small (<2% of the total). This was a result of variability in the cartridge geometry after holes were drilled into the tops to allow the free flow of sampled air through the cartridge. The cartridge tops, made from Millipore PetriSlide coverings, were modified by drilling holes in them and removing the excess material from their edges. This was done by hand, and non-uniformity in their construction is certain. Many of the edges of the drilled holes blocked the camera's view of the cartridge bottom; therefore, variability in their placement would have caused non-uniform blocking of the camera's view.

Variability was also caused by dimpling in the aluminum foil covering and the mesh bottoms in each cartridge. Corn samples were shaken for 15 s after being covered, and the corn striking the covering caused dimpling in the foil. This dimpling created diffuse reflection that may have not been uniform between corn samples. Additionally, the metal mesh discs placed in the bottom of the cartridges were discolored by small amounts of oxidation as a result of repeated washings. This discoloration may have been substantial enough to cause some of the pixels representing mesh in the acquired images to have a value lower than the segmentation threshold (in this study, the lower threshold was 70). During testing, all sources of lighting excluding the overhead room lights were covered. The overhead lights did not change location, and it is assumed that their output was consistent over the test period. It is doubtful that the lighting conditions caused the large variability in the blank replications, but it is possible.

The adaptation of this system to a handheld device would require the development of an automated calibration method or controlling for physical cartridge and lighting differences. For example, molding or other reproducible manufacturing method would reduce the physical differences between cartridges, currently modified individually by hand. A custom cartridge design would dispose of the need for the mesh bottom used to prevent *M. croceipes* from crawling out the bottom. The cartridge would need to be made from Teflon, a hard plastic, or other inert material. Additionally, enclosing the system and using a fixed light source would reduce possible variability in lighting and shadows. However, for this study, the cartridge/lighting variability was removed from the control and test treatments by calibrating each image set before statistical comparisons were made.

Effects of Treatment and Dosage on Response

The control and test treatments (180 observations) were calibrated and analyzed to determine the effects of treatment and dosage on the mean response (average integration values over 60 s test period) (figs. 5 and 6). Figure 5 shows the control and test treatment mean responses grouped by dosage. The errors bars were calculated using $n = 5$ and $\alpha = 0.05$ for each treatment per dosage. The response of the *M. croceipes* groups over the 60 s test period can be seen in figure 6. The controls for all dosages were tightly grouped and were similar to the test treatment at the 0.01 mg (111 μg L^{-1}) dosage. The test treatments at the 0.5 mg (5.5 mg L^{-1}) and 0.1 mg (1.1 mg L^{-1}) dosages were both significantly different from all other treatment/dosage pairs after, at most, 20 s. Errors were calculated using $n = 5$ and $\alpha = 0.05$ for each treatment per dosage.

Effects of Control and Test Treatments on Response

Five groups of *M. croceipes* (five individuals per group) received both control and test treatments using 0.5 mg of 3-octanone. The behavioral response of *M. croceipes* at the 0.5 mg (5.5 mg L^{-1}) dosage level was significant across treatments (d.f. = 1, $n = 120$, $p < 0.0001$). The mean response of the test treatment (2.8 pixel*s) was significantly higher than that of the control treatment (0.5 pixel*s) (fig. 5). The time (d.f. = 11, $n = 120$, $P < 0.0001$) and treatment*time interaction (d.f. = 11, $n = 120$, $p < 0.0001$) effects were also both significant, indicating that the integration values were

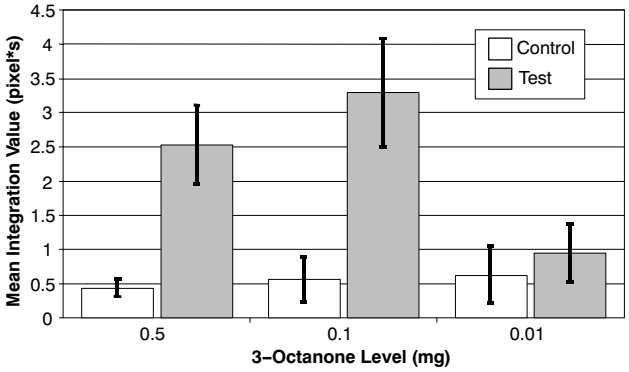


Figure 5. Response for each treatment per dosage level and 95% confidence intervals. The means of the controls are not significantly different from each other or the test treatment at 0.01 mg (111 mg L^{-1}). The means of the test treatments at 0.5 and 0.1 mg (i.e., 5.5 and 1.1 mg L^{-1} respectively) are not significantly different from each other but are different from the control treatments and the test treatment at 0.01 mg.

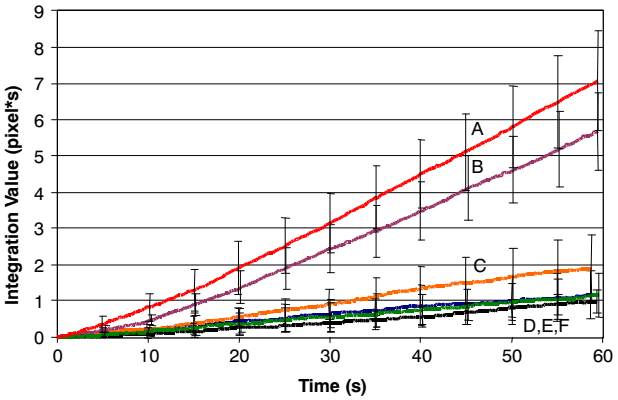


Figure 6. Means and 95% confidence intervals for all treatments at all dosage levels. Trends A, B, and C are the mean response to 0.1 mg (1.1 mg L^{-1}), 0.5 mg (5.5 mg L^{-1}), and 0.01 mg (111 mg L^{-1}) dosage levels, respectively. Trends D, E, and F are the mean response to control treatments. Responses A and B were measured as significantly different from test response C and all control responses (D, E, F) after 20 s.

dependent on both treatment and elapsed time. The system was able to detect a significant difference in the test and control treatment responses in approximately 10 s (fig. 6).

Similar results were obtained with five groups of *M. croceipes* (five individuals per group) receiving both control and test treatments using 0.1 mg of 3-octanone. The behavioral response of *M. croceipes* at the 0.1 mg (1.1 mg L^{-1}) dosage level was significant across treatments (d.f. = 1, $n = 120$, $p = 0.0002$). The mean response of the test treatment (3.58 pixel*s) was significantly higher than that of the control treatment (0.61 pixel*s) (fig. 5). The time (d.f. = 11, $n = 120$, $p < 0.0001$) and treatment*time (d.f. = 11, $n = 120$, $p < 0.0001$) effects were also both significant, indicating that the 120 integration values (12 obs/rep for five test and five control reps) were time and treatment dependent. The system was able to detect a significant difference in the test and control treatment responses in approximately 10 s (fig. 6).

The system was able to quantify the behavior of the trained wasps in such a way as to successfully distinguish between the crowding behavior exhibited when presented with the target odor at the 0.5 and 0.1 mg levels and the individual searching behaviors exhibited when presented with only the odor of the corn (fig. 5). When looking at

individual dosages, a significant difference in the two treatments was detectable in as little as 10 s (fig. 6). When the results from the dosages are pooled, a significant difference between the tests and controls was detectable in approximately 20 s.

The behavioral response of *M. croceipes* at the 0.01 mg (111 μg L^{-1}) dosage level was not significantly different across treatments (d.f. = 1, n = 120, p = 0.31) (fig. 5). However, the time (d.f. = 11, n = 120, p < 0.0001) and treatment*time interaction (d.f. = 11, n = 120, p = 0.04) effects were both significant at $\alpha = 0.05$, indicating that the 120 integration values (12 obs/rep for five test and five control reps) were time dependent (fig. 6). At this dosage, it appears that the odor concentration was too low to elicit a crowding behavior strong enough for the system to detect as significantly different from the control, or the wasps were unable to detect the odor.

Effects of Dosage Levels on Treatment Response

Fifteen groups of *M. croceipes* (five individuals per group) received control (corn odor only) treatments before receiving test treatments at one of the three dosages. Dosage had no significant effect on *M. croceipes* response to the control treatment (12 obs/rep, five reps, three doses) (d.f. = 2, n = 180, p = 0.7159) (fig. 6). Dosage*time interaction effects were not significant (d.f. = 22, n = 180, p = 1.0), but time effects were significant (d.f. = 11, n = 180, p < 0.0001), indicating that the integration values were affected by time but not by what test treatment dosage they preceded.

These results imply that the groups of wasps exhibited similar searching behaviors. No group spent significantly more or less time within the ROI than any other group, allowing for the assumption that test treatment results were not biased by the normal searching behavior of the trained *M. croceipes*.

Dosage did have a significant effect on *M. croceipes* response to the test treatment (12 obs/rep, five reps, three doses) (d.f. = 2, n = 180, p = 0.0005). The 0.1 mg (3.58) and 0.5 mg (2.76) response means were not significantly different from each other, but they were both significantly different from the 0.01 mg response mean (1.03) (fig. 6). Both time (d.f. = 11, n = 120, p < 0.0001) and the dosage*time interaction (d.f. = 22, n = 120, p < 0.0001) significantly affected the integration values.

The system was not able to distinguish between responses to dosages that were significantly different from the controls. Therefore, it appeared that the concentration of the target odor could not be inferred from the magnitude or slope of the response curve. Additional investigation was needed to infer target odor concentration levels from *M. croceipes* behavioral responses.

Conclusion

A computer vision system with Visual Cortex image analysis software was successfully utilized to objectively observe and quantify the crowding behavior of five trained female *M. croceipes* parasitoid wasps. The software was able to distinguish between the strong crowding behavior exhibited by trained *M. croceipes* presented with the target odor (3-octanone) at the 1.1 and 5.5 mg L^{-1} concentrations and the random searching observed during the control treatment within 15 s. The same did not hold true when the wasps were exposed to 3-octanone at 111 μg L^{-1}. The inability to discriminate at the lowest concentration could be a function of the conditioning concentration used to condition the wasp to 3-octanone, the lower limits of the wasp detection abilities, and/or the limitations in measuring subtle changes in behavior when the wasp detects small concentrations of odors. The wasps were conditioned using 3-octanone at 5.5 mg L^{-1}, and future studies should investigate the level of detection when conditioning *M. croceipes* at different dosages of target chemical. In addition, detection limits could be related to actual fungal levels detected using enzyme-linked immunoassay (ELISA) in corn and peanuts. Future work will also investigate the development of mathematical algorithms that interpret the subtle movement of individual *M. croceipes*. It is possible that *M. croceipes* exhibit behavioral movements that occur before crowding. Understanding behavioral or physiological changes temporally closer to the neurological response of *M. croceipes* will allow for increased performance when detecting their response to target odors.

References

Balch, T., Z. Khan, and M. Veloso. 2001. Automatically tracking and analyzing the behavior of live insect colonies. In *Proc. 5th Intl. Conf. on Autonomous Agents 2001*, 521-528. New York, N.Y.: ACM Press.

Lewis, W. J., and R. L. Burton. 1970. Rearing *Microplitis croceipes* in the laboratory with *Heliothis zea* as hosts. *J. Econ. Entomology* 63(1): 656-658.

Olson, D. M., G. C. Rains, T. Meiners, K. Takasu, M. Tertuliano, J. H. Tumlinson, F. L. Wäckers, and Lewis. 2003. Parasitic wasp learn and report diverse chemicals with unique conditionable behaviors. *Chem. Senses* 28(6): 45-549.

Parente, P. 2003. User solution: Webcam image acquisition. In *Image Acquisition and Processing with LabView*, 65-58. C. G. Relf, ed. Boca Raton, Fla.: CRC Press.

Shapiro, L. G., and G. C. Stockman. 2001. *Computer Vision.* 1st ed. Englewood Cliffs, N.J.: Prentice Hall.

Takasu, K., and W. J. Lewis. 1993. Host and food-foraging of the parasitoid, *Microplitis croceipes*: Learning and physiological state effects. *Biol. Control* 3(1): 70-74.

Tertuliano, M., D. M. Olson, G. C. Rains, and W. J. Lewis. 2004. Influence of handling and conditioning protocol on learning and memory of *Microplitis croceipes. Entomologia Experimentalis et Applicata* 110(2): 165-172.

Wäckers, F. L., C. Bonifay, and W. J. Lewis. 2002. Conditioning of appetitive behavior in the Hymenopteran parasitoid *Microplitis croceipes. Entomologia Experimentalis et Applicata* 103(2): 135-138.